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AD-A228 465

CONTRACT NO: DAMD17-85-C-5232

TITLE: GENETICALLY-ENGINEERED POXVIRUSES AND THE CONSTRUCTION OF LIVE RECOMBINANT VACCINES

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REPORT DATE: August 1, 1990

TYPE OF REPORT: Annual and Final Report

PREPARED FOR: U.S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND
Fort Detrick, Frederick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;
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SECURITY CLASSIFICATION OF THIS PAGE

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1a. REPORT SECURITY CLASSIFICATION Unclassified	1b. RESTRICTIVE MARKINGS
2a. SECURITY CLASSIFICATION AUTHORITY	3. DISTRIBUTION/AVAILABILITY OF REPORT Approved for public release; distribution unlimited
2b. DECLASSIFICATION/DOWNGRADING SCHEDULE	
4. PERFORMING ORGANIZATION REPORT NUMBER(S)	5. MONITORING ORGANIZATION REPORT NUMBER(S)
6a. NAME OF PERFORMING ORGANIZATION Albany State Health Laboratory 6b. OFFICE SYMBOL (If applicable)	7a. NAME OF MONITORING ORGANIZATION
6c. ADDRESS (City, State, and ZIP Code) Albany, NY 12201	7b. ADDRESS (City, State, and ZIP Code)
8a. NAME OF FUNDING/SPONSORING ORGANIZATION U.S. Army Medical Research & Development Command 8b. OFFICE SYMBOL (If applicable)	9. PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER Contract No. DAMD17-85-C-5232
8c. ADDRESS (City, State, and ZIP Code) Fort Detrick Frederick, Maryland 21702-5012	10. SOURCE OF FUNDING NUMBERS PROGRAM ELEMENT NO. 62787A; PROJECT NO. 3M1 62787A871; TASK NO. AD; WORK UNIT ACCESSION NO. 364

11. TITLE (Include Security Classification)
Genetically-Engineered Poxviruses and the Construction of Live Recombinant Vaccines

12. PERSONAL AUTHOR(S)
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13a. TYPE OF REPORT	13b. TIME COVERED	14. DATE OF REPORT (Year, Month, Day)	15. PAGE COUNT
Annual & Final Report	FROM 7/15/85 TO 7/14/90	1990 August 1	

16. SUPPLEMENTARY NOTATION
Annual covers the period July 15, 1988 - July 14, 1990

17. COSATI CODES			18. SUBJECT TERMS (Continue on reverse if necessary and identify by block number)
FIELD	GROUP	SUB-GROUP	Lab Animals; Mice; Gene Cloning; Vaccines; Poxviruses; Recombinant DNA; RA I; Vaccinia; BD
06	02		
06	13		

19. ABSTRACT (Continue on reverse if necessary and identify by block number)

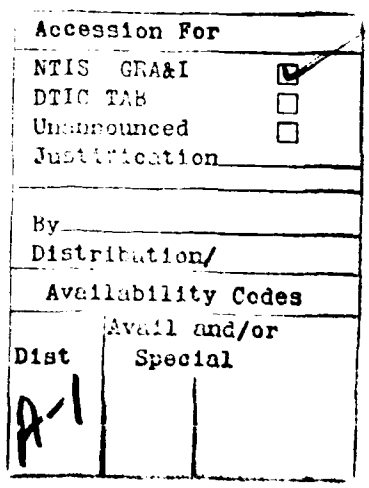
A survey of the transcript levels associated with vaccinia virus was performed. Specific and unique vaccinia promoters were identified by standard techniques and manipulated to drive the expression of foreign genes such as Beta lactosidase and glycoproteins. Promoters of all three classes (early, intermediate, late) were identified and studied. No single promoter was found to be exceptional. Most strong promoters C1OLW, H6, 11K, IL3 gave good expression which fell within several fold of each other. No promoter was found that gave magnitude increased levels of expression. Generally, irrelevant proteins such as Beta galactosidase could be expressed at high levels sufficient to visualize on Coomasie stained gels. Unfortunately, this was not the case when relevant proteins such as viral glycoproteins were expressed. The "additional" factors that need to be involved for high level expression by vaccinia virus vectors are still unknown.

20. DISTRIBUTION/AVAILABILITY OF ABSTRACT	21. ABSTRACT SECURITY CLASSIFICATION
☐ UNCLASSIFIED/UNLIMITED ☐ SAME AS RPT ☐ DTIC USERS	Unclassified
22a. NAME OF RESPONSIBLE INDIVIDUAL Mrs. Virginia M. Miller	22b. TELEPHONE (Include Area Code) (301)663-7325 22c. OFFICE SYMBOL SGRD-RMI-S

DD Form 1473, JUN 86 *Previous editions are obsolete.* SECURITY CLASSIFICATION OF THIS PAGE

FOREWORD

In conducting research using animals, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (NIH Publication No. 86-23, Revised 1985).



The Research Objectives of the Research Contract #DAMD17-85-C-5232 BASIC were twofold:

i) To genetically engineer poxvirus vectors to optimize the expression of foreign gene(s) pertinent to the construction of live recombinant vaccines.

ii) To genetically engineer poxvirus vectors to further attenuate the virus resulting in a vaccine vector that is safer than any currently available vaccine strain.

SUMMARY

A) A survey of the transcript levels associated with vaccinia virus was performed. Specific and unique vaccinia promoters were identified by standard techniques and manipulated to drive the expression of foreign genes such as Beta lactosidase and glycoproteins. Promoters of all three classes (early, intermediate, late) were identified and studied. No single promoter was found to be exceptional. Most strong promoters C10LW, H6, 11K, IL3 gave good expression which fell within several fold of each other. No promoter was found that gave magnitude increased levels of expression. Generally, irrelevant proteins such as Beta galactosidase could be expressed at high levels sufficient to visualize on Coomasie stained gels. Unfortunately, this was not the case when relevant proteins such as viral glycoproteins were expressed. The "additional" factors that need to be involved for high level expression by vaccinia virus vectors are still unknown.

Significantly, however, with regard to vaccine formulation the selection of the proper temporally regulated promoter element may be more critical than the actual level of protein expression.

B) The genetic engineering of the virus to construct an attenuated vector has been carried out in several ways with overall significant success.

i) In order to genetically stabilize, i.e. to free the genome thus rendering it less susceptible to mutations, we looked for and investigated the genetic functions responsible for recombination. A genetic analysis of temperature sensitive mutants was performed to determine the genes involved in recombination. An unexpected finding was that the vaccinia virus DNA polymerase was involved in recombination at a level other than DNA synthesis and replication. This fact obviously complicates manipulation of the vaccinia virus recombination functions since the DNA polymerase is an essential viral gene.

ii) A DNA ligase function with obvious implications in recombination was identified. It was shown to be an early protein. Genetic manipulation revealed that it was not an essential gene for the virus. This unexpected result reveals an unprecedented complexity in the recombinational machinery of poxviruses.

iii) A number of regulatory genes responsible for host cell restriction of growth in tissue culture were identified and manipulated. These genes specifying viral replication in tissue culture cells of human, porcine and rabbit cells were described. Manipulation

of these genes, for example differential deletion analysis, revealed that attenuation of the virus could be affected without significantly diminishing the ability of the virus to act as a vector for vaccination purposes.

Manipulation of the host range genes also provided for the cloning and expression of foreign genes in vaccinia virus vectors that greatly simplified and accelerated the construction and analysis of vaccinia recombinants.

iv) In order to set a baseline and to have available the organizational information of the genetic basis of the vaccinia virus the entire DNA genome was sequenced. This analysis revealed that vaccinia virus has 198 open reading frames and 191,636 base pairs in the entire genome. This information will be invaluable for understanding the biology of the virus.

v) Partly due to the availability of the DNA sequence, a number of genes have been deleted from the virus. Many of these genes have been implicated in undue virulence. Hence such manipulations have resulted in greatly attenuated vaccinia virus strains that have been shown in preclinical studies to retain their efficacy for vaccination purposes. To date, more than sixty (60) genes have been deleted from the virus. Significantly, such an analysis has allowed us to manipulate (genetically) vaccinia virus such that it is now susceptible to antiviral intervention via conventional, well defined and therapeutically acceptable methods.

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PATENT APPLICATION

The contract support was noted in a filed patent application,
"Recombinant Poxvirus Host Range Selection System."

PERSONNEL

Personnel receiving support from the contract were:

Dr. Elizabeth Norton
Dr. Gerard Johnson